Bioinformatics in Type 1 Diabetes: Oxidative Stress and Complications

Timothy D. Wiggin*, MS, Kelli A. Sullivan*, PhD, Matthias Kretzler**, MD, Eva L. Feldman*, MD PhD
Departments of Neurology* and Internal Medicine**, University of Michigan, Ann Arbor, Michigan, USA

Abstract

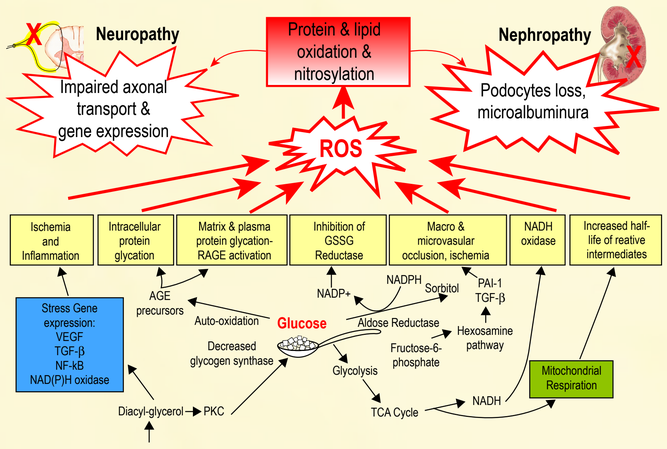
The National Center for Integrative Biomedical Informatics (NCIBI) was funded by the NIH in 2005 to develop tools that allow researchers to integrate and understand the enormous quantity of information available to them. While most of the Center researchers are computationally oriented, the Driving Biological Problems (DBPs) provide both a target and a test bed for their tools.

One of these DBPs is understanding and finding treatments for the complications of Type I Diabetes Mellitus. The specific aims of this DBP are to understand the link between oxidative stress caused by excess glucose and its adverse effects on cellular function and cell death in tissues prone to diabetic complications.

The NCIBI and the Type I Diabetes DBP collaborate in four main areas:
1) DNA microarray analyses
2) Metabolic and regulatory network modeling
3) Biomarker prediction through pathway modeling
4) Natural language processing

Introduction

Animal and *in vitro* experiments implicate a number of enzymatic and non-enzymatic pathways of glucose metabolism in the initiation and progression of complications. Recently a link has been established that provides a unified mechanism of tissue damage. Cellular pathways become perturbed as a direct or indirect consequence of hyperglycemia-mediated superoxide overproduction by the mitochondrial electron transport chain. This increase in reactive oxygen species (ROS) reflects an overall increased state of cellular oxidative stress. Inhibition of ROS or maintenance of euglycemia restores metabolic and vascular balances and blocks both the initiation and progression of complications.



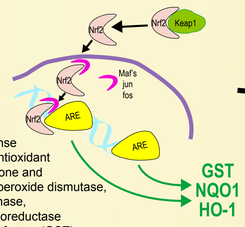
The purpose of linking the JDRF Center for the Study of Complications in Diabetes and the NCIBI group at the University of Michigan is to apply informatics tools to the study of type 1 diabetes including:
- DNA microarray analyses - to discover genes regulated by oxidative stress
- Pathway mapping - to predict protein interactions
- Natural language processing tools - to better search the published literature

Methods

Messenger RNA and protein are extracted from cells and tissues and analyzed via western immmunoblotting and/or DNA microarray. Regulation of the proteins and genes in question are then compared with the literature and gene and protein databases using the following tools:
- ChipInspector – Screen for non-specific binding and false positives
- Bibliosphere – Literature mining and promoter modeling
- MiMI – Protein interaction discovery and modeling
- GeneGo – Metabolic and regulatory network modeling
- Ingenuity – Link microarray data to KEGG pathways
- Bayesian Network analysis – Biomarker prediction through pathway modeling
- Molecular Modeling Database – Structure analysis to identify binding sites
- Gene Expression Omnibus – Comparison to previous array experiments

Overview

Hypothesis



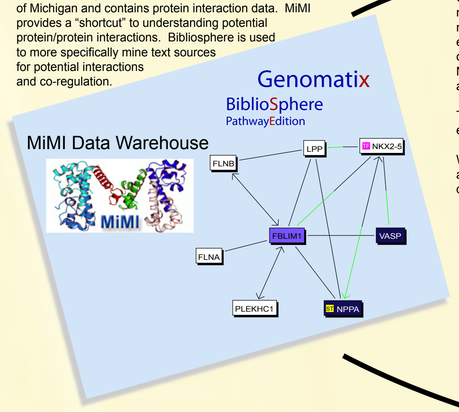
Cellular oxidative defense mechanisms include antioxidant molecules e.g. glutathione and enzymes including; superoxide dismutase, catalase, heme-oxygenase, NAD(P)H:quinone oxidoreductase and glutathione S-transferase (GST).

The cytoplasmic factor Nrf2, regulates the expression of these enzymes by binding to the antioxidant response element (ARE).

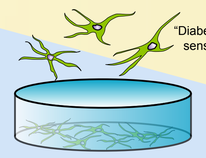
We hypothesize that activation of the ARE will increase the antioxidant response, decrease ROS and prevent cellular damage.

Informatics Tools

Informatics tools are used to explore and confirm hypotheses. MiMI is a biological data warehouse developed at the University of Michigan and contains protein interaction data. MiMI provides a "shortcut" to understanding potential protein/protein interactions. Bibliosphere is used to more specifically mine text sources for potential interactions and co-regulation.

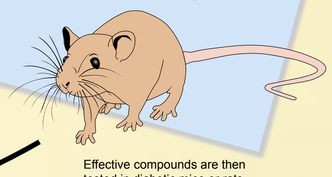


Cell Culture Experiments



"Diabetes in a dish." Primary cultures of sensory neurons are used to examine the impact on ARE activation and neuron survival by potential therapeutic compounds.

Whole Animal Experiments



Effective compounds are then tested in diabetic mice or rats.

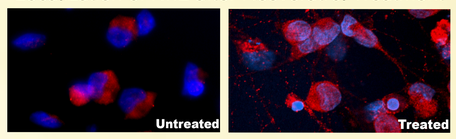
DNA Chip Analysis

DNA chip technology examines the expression of thousands of genes from both cell culture and whole animal experiments. These analyses are used to confirm hypotheses and discover new genes of interest.

When connected with biomarkers and physiological data, DNA chips reveal a more complete picture of the disease process and treatment effects.

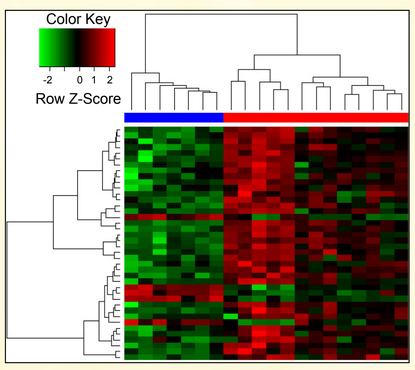
Results

Localization of Nrf2 after Resveratrol Treatment



Nrf2, a protein involved in GST regulation, translocates from the cytosol (left panel) to the nucleus (right panel) following resveratrol treatment.

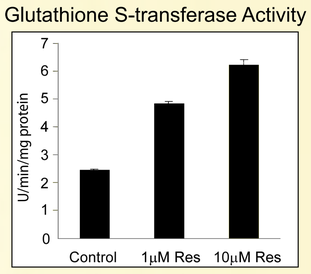
DNA Chip Analysis



A DNA chip experiment was performed comparing gene expression in normal patients and those with early diabetic nephropathy (kidney damage).

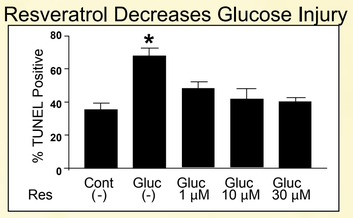
It was found that glutathione S-transferase (GST) was differentially regulated in diabetic patients.

Glutathione S-transferase Activity



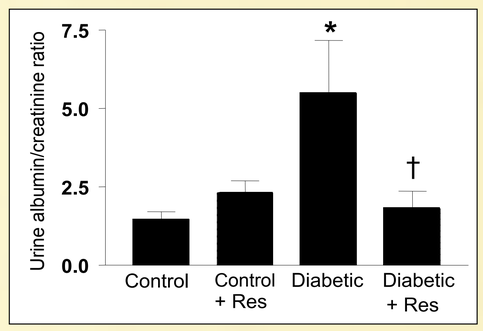
Primary Dorsal Root Ganglion (DRG) neurons were exposed to resveratrol (Res), a botanical with reported antioxidant effects. Resveratrol increased GST activity in DRG neurons.

Resveratrol Decreases Glucose Injury



Pretreating neurons with resveratrol (Res) reduces programmed cell death in response to hyperglycemia (Gluc).

Effect of Resveratrol on Kidney Function



Diabetic mice were treated with resveratrol (Res) to determine its effectiveness *in vivo*. Resveratrol prevented diabetic nephropathy and returned albumin/creatinine ratios to those observed in normal mice.

Conclusions

Experiments investigating the expression, activity and localization of proteins involved in the antioxidant response are in progress. Data collection and analyses are enhanced by the informatics tools available to the laboratory via the NCIBI. Potential protein interactions and gene regulation data are essential to confirm and explore new hypotheses.

Acknowledgements

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